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RC 261. A1 A46

NOS

From: Holleran, Anne
Sent: Tuesday, September 04, 2001 6:03 PM
To: STIC-ILL
Subject: refs. for 09/251,133

Examiner: Anne Holleran
Art Unit: 1642; Rm 8E03
Phone: 308-8892
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362507

Please send me copies of the following :

1. Chien, J. et al. Mol. and Cell. Endocrinology (2001) 181(1-2): 69-79
2. Chien, J. et al. Int. J. of Cancer (2001) 91(1): 46-54
3. Chien, J. et al. Oncogene (1999) 18(22): 3376-3382
4. Wong, E.C.C. et al. Proc. Amer. Assoc. for Cancer Res. (1997) 38: 288
5. Rayford, W. et al. Prostate (1997) 30(3): 160-166
6. Xue-Zhang, Q. et al. Endocrine (1995) 3(6): 445-451
7. Shah, G.V. et al. Endocrinology (1994) 134(2): 596-602
8. Rayford, W. et al. J. of Urology (1994) 151(5 suppl): 490A
9. Rayford, W. et al. J. of Urology (1993) 149(4 suppl): 479A
10. Shah, G.V. et al. Prostate (N.Y.) (1992) 21(2): 87-97
11. Sagol, O. et al. Annals of Medical Sciences (1999) 8(1): 14-21
12. Sussenot, O. et al. Prostate (1998) 36(suppl. 8): 43-51
13. Hanna, F.W. et al. J. Endocrinol. (1997) 152(2): 275-281
14. Sim, S.J. et al. Annals of Clinical and Laboratory Science (1996) 26(6): 487-495
15. Watanabe, K. et al. Fukushima J. Medical Science (1995) 41(2): 141-152
16. Esik, O. et al. European J. Gynaecological Oncology (1994) 15(3): 211-216

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BIOLOGY 13

invasiveness and poor prognosis. Recent experimental evidence suggests that downstream activation of PLC γ 1 by growth factor receptor-tyrosine kinases (GFR-TK) such as EGFR, PDGFR and IGFR mediates cell motility as a process separate from cell proliferation. Here we investigate the effect of inhibition of PLC γ 1 activity on GBM invasion of fetal rat brain aggregates (FRBAs) in a spheroid co-culture system. We used a specific PLC γ 1 inhibitor (U-37122) and a dominant negative PLC γ 1 fragment (SH2 and SH3 domains; amino acids 517-901) which inhibits PLC γ 1 activation by GFR-TKs. U-37122 at 2 μ M caused a near-complete inhibition of invasion of a human GBM cell line overexpressing wild-type EGFR. Transfection of the C6 rat GBM cell line with a dominant negative gene fragment (PLC γ 2) encoding the above described PLC γ 1 fragment also abrogated growth factor stimulated cell motility and invasion of FRBAs. This coincided with a significant decrease in PLC γ 1 associated tyrosine phosphorylation. These results suggest a role for PLC γ 1 in mediating growth factor induced C6 cell migration and invasiveness.

#1932 PC-3M cells transfected with a GTPase deficient Gs alpha mutant protein have increased invasive potential. Wong, E.C.C., Chien, J., Croughan, W., Pantazis, C.G., Noble, M.J., and Shah, G.V. *University of Kansas Medical Center, Kansas City, KS 66160*

In order to test the hypothesis that elevated intracellular cAMP in prostate cells results in increased metastatic potential, we transfected PC-3M cells with either wild type Gs alpha cDNA (GSWT) or a mutant Gs alpha cDNA (GSA) coding for a Gs alpha protein with deficient GTPase activity. cAMP levels and tritiated thymidine incorporation was 7-8 fold and 34% higher, respectively, in the GSA versus GSWT cells. Use of Matrigel invasion chambers to assess *in vitro* invasive potential showed that GSA cells demonstrated 20-30 fold higher invasiveness than GSWT cells. Chemoattractant medium containing conditioned medium from GSA cells resulted in 50% higher invasiveness than chemoattractant medium containing conditioned medium from GSWT cells. In contrast, GSWT cells demonstrated little invasion using chemoattractant medium containing conditioned medium from either transfected cell line. Calcitonin has been shown to stimulate proliferation in prostate cancer cells. Use of anti-calcitonin-like (CT) peptide antibody reduced invasion by 63% in a dose dependent fashion in GSA cells. These experiments suggest that increased cAMP levels in PC-3M cells results in increased intrinsic invasive potential which is augmented by prostate CT or other related factors.

#1933 Migration of invasive breast cancer cells is controlled by a G-protein linked receptor signaling pathway and integrin activation. Popper, G., and Quaranta, V. *Department of Cell Biology, The Scripps Research Institute, 10550 N. Torrey Pines Road, La Jolla CA 92037 USA*

Invasion by breast cancer cells requires migration across mammary gland basement membrane. Both malignant (MCF-7) and non-malignant (HUMEC, MCF-10A) mammary epithelial cells bind the mammary basement membrane protein laminin-5 (Ln-5) via the α 3 β 1 integrin. However, MCF-7 cells can be distinguished from HUMEC and MCF10-A cells by their ability to constitutively migrate on Ln-5 in haptotactic migration assays in the absence of serum or chemotactic factors. We have identified a signaling pathway that controls migration in breast epithelial cells, and that includes the epidermal growth factor receptor, cholera toxin- and pertussis toxin-sensitive G-proteins, adenylyl cyclase, cAMP dependent protein kinase (PKA), and antibody-activated α 3 β 1 integrin. In non-malignant cells, activation of this pathway at any of these levels stimulates migration on Ln-5. In malignant cells, inhibition of this pathway with the PKA inhibitor H-89 or function-blocking antibodies against α 3 β 1 integrin blocks constitutive migration on Ln-5. These results suggest that a G-protein signaling pathway may play an important role in regulating mammary epithelial cell behavior and that abnormal, constitutive activation of this pathway may induce cell migration during invasion by breast cancer cells.

#1934 Chemoattraction of PC-3 prostatic cancer (PCa) cell line by proteins secreted in HOS TE85 (TE85) human osteoblastic osteosarcoma cells. Gygi, C.M., Sutkowski, D.M., and Neubauer, B.L. *Service of Urology, Centre Hospitalier Universitaire Vaudois, 1011 Lausanne, Switzerland [C.M.G., Lilly Research Laboratories, Indianapolis, IN 46285]*

Approximately 70% of PCa patients will develop osseous metastases. We evaluated the chemoattractive role of (TE85) bone-secreting proteins on the human prostatic cancer cell line PC-3. TE85 serum-free conditioned media was dialyzed and proteins >3.5 kD were concentrated. Various concentrations of conditioned media was used as chemoattractant in chemotaxis studies and screened using western blots. Specific purified proteins were used to evaluate their individual role(s) in chemoattraction. *In vitro* chemoattraction assays were performed using transwell chambers with a 8 μ m pore polyethylene terephthalate membranes. Proteins from TE85 conditioned media attracted PC-3 cells in a concentration dependent manner. Chemoattraction assays with purified proteins EGF, and TGF- α showed significant stimulation of migration of PC-3 cells. EGF and TGF- α are potent chemoattractants secreted by bone that stimulate migration of PCa cells. Other growth factors/peptides such as bFGF, NGF, TGF β , IGF I/II and PTHLP were not active as chemoattractants for PC-3 cells. To the extent that TE85 human osteoblastic osteosarcoma cells express normal human bone

proteins, a factor in the etiology of PCa metastasis to bone is a function of these chemoattractant properties in addition to the mechanic dissemination due to vascular drainage.

#1935 Dunning prostate-derived motility factor induces haptotactic migration of MAT-LyLu cells. Krasij, R., Teubel, W.J., Schröder, F.H., and Romijn, J.C. *Department of Urology, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, the Netherlands*

In the past decades, a number of Dunning R3327 rat prostatic tumor sub-lines have been established that differ in androgen sensitivity, differentiation state, and metastatic potential. We use the sub-lines G (well-differentiated, non-metastatic), AT2.1 (anaplastic, non-metastatic), and MAT-LyLu (anaplastic, metastatic), as a cell panel to investigate cellular changes that lead to metastatic behavior of cancer cells. Previously, we have shown that conditioned media from AT2.1 and MAT-LyLu cells induce migration of MAT-LyLu cells in a modified Boyden chamber migration assay, whereas G cell conditioned medium does not contain this Dunning prostate-derived motility factor (DPMF). Now we report that DPMF is a trypsin-sensitive protein factor that induces haptotactic migration of MAT-LyLu cells. Furthermore, RGD-containing peptides specifically inhibit DPMF-induced migration. These results suggest that DPMF is an extracellular matrix component-like factor. Bioactive, partially purified fractions, obtained by Concanavalin A and anion exchange chromatography, showed several bands on SDS-PAGE with molecular weights ranging from 200 to 250 kD. Further purification and subsequent protein sequencing will eventually reveal the nature of DPMF.

#1936 Identification of HGF/Met regulated genes: Implications for HGF/Met-mediated cell invasion and metastasis. Taylor, G.A., Jeffers, M., Webb, C.P., and Vande Woude, G.F. *ABL-Basic Research Program, NCI-Fredrick Cancer Research and Development Center, P.O. Box B, Frederick, MD 21702*

Hepatocyte growth factor (HGF) is a pleiotropic effector of cells expressing its receptor, the Met tyrosine kinase. While HGF expression is normally restricted to mesenchymal cells and Met expression to epithelial cells, coexpression of HGF and Met in mesenchymal cells can lead to changes in cellular morphology *in vitro*, formation of branching structures in matrigel cultures, and increased tumorigenic, invasive, and metastatic capabilities in nude mice. We have begun to address the molecular basis for these phenomena, by using differential display screening to identify changes in gene expression in SK-LMS-1 human leiomyosarcoma cells that were engineered to coexpress HGF and Met. We noted decreases in fibronectin and transforming growth factor β expression and increases in integrin α 5 expression. Similar changes were noted in other human tumors and transformed mouse fibroblasts that also contained an HGF/Met autocrine loop. Our results define changes in cell-extracellular matrix interactions that underlie HGF/Met-mediated cell motility and migration. Research sponsored by the National Cancer Institute, DHHS, under contract with ABL.

#1937 Expression of motility cycle components *in vivo* reflects metastatic potential of human tumor xenografts. Timár, J., Ráabó, E., Fazakas, Z., Raz, A., and Honn, K.V. *1st Institute of Pathology and Experimental Cancer Research, Semmelweis University of Medicine, Budapest, Hungary, Karmanos Cancer Institute, Department of Radiation Oncology, Wayne State University, Detroit, MI*

Previous studies on the regulation of tumor cell motility *in vitro* indicated that the autocrine motility is mediated by Autocrine Motility Factor, its receptor gp78 and a signaling pathway involving the function of 12-lipoxygenase and PKC. We have tested by immunocytochemistry the expression of the components of this regulatory pathway (gp78, platelet-12-lipoxygenase and PKC) *in vivo* in primary tumors of human melanoma (HT168, HT168-M1, HT199) and prostate carcinoma (PC3 and DU-145) xenografts. Spontaneous metastatic potential was detected in newborn rats in case of melanoma and in SCID mice in case of prostate carcinomas implanted orthotopically. Flow cytometry indicated that gp78 receptor expression is universally upregulated in metastatic tumor cells associated with PKC α upregulation. Furthermore, it was possible to detect upregulation of 12-lipoxygenase expression in metastatic HT168 melanoma and DU-145 prostate carcinoma. These data suggest that the expression of motility cycle components in the primary tumors may be useful marker of the actual metastatic potential of some human tumor types. This work was supported by the following grants: NATO CRG 950287, Fogarty TW285, Hungarian NSF T21149, NIH CA-47115, CA 29997 and CA-51714.

#1938 Absence of functional CD44 hyaluronan receptor on human NMYC amplified neuroblastoma cells. Gross, N., Böhm, K., and Beretta Brognara, C. *Onc-hematology Unit, Pediatrics, University Hospital, CHUV, 1011 Lausanne, Switzerland*

CD44 represents a group of surface glycoproteins, involved in leukocytes homing and activation, and tumor metastasis. CD44 has been shown to be the major receptor for hyaluronan (HA) and most of CD44 known functions are attributed to its ability to recognize HA. Whereas overexpression of the CD44 standard molecule and particular spliced isoforms has been linked to tumor growth and metastasis in several adult tumors, we have demonstrated that in human neuroblastoma (NB), a childhood cancer, high stages and NMYC amplified tumors failed to express CD44. Lack of CD44 expression has been shown to be strongly associated to NMYC amplification (NMA) and to represent another pow-